In the Specification:

Please amend the specification as shown:

Please delete the paragraphs on page 11, lines 4-23 and replace it with the following paragraph:

Figure 1 is a schematic diagram illustrating two strategies using circular permutation and domain insertion for generating libraries of molecular switches according to the invention **(GSGGG linker disclosed as SEQ ID NO: 1)**.

Figure 2 illustrates steps in creating a cyclized gene using a DKS linker according to the invention **(SEQ ID NOS 78-84, respectively in order of appearance)**.

Figure 3 illustrates steps in creating a cyclized gene using a GSGGG linker **(SEQ ID NO: 1)** according to the invention **(SEQ ID NOS 85-91, respectively in order of appearance)**.

Figure 4 is a diagram illustrating steps in preparing an acceptor DNA sequence for insertion of an insertion DNA sequence at a specific site in the acceptor DNA sequence according to the invention **(SEQ ID NOS 92-97, and 94, respectively in order of appearance)**.

Figures 5A-G are schematic diagrams depicting several applications of the molecular switches of the invention **(oligonucleotide disclosed as SEQ ID NO: 98)**.

Figures 6A-C illustrate a novel fusion molecule comprising sequences from an effector protein (maltose binding protein, MBP) and an enzyme (β-lactamase, BLA) according to an aspect the invention. Figure 6A shows the steps involved in creating the fusion molecule. Figure 6B is a schematic diagram illustrating the amino acid sequence of the fusion protein, termed RG13. Figure 6C is a drawing illustrating the structure of the RG13 fusion protein **(GSGGG linker disclosed as SEQ ID NO: 1)**.

Please delete the paragraph on page 12, lines 9-12 and replace it with the following paragraph:

Figure 11 is a schematic diagram illustrating a strategy for creating a library in which a circularly permuted *bla* gene is inserted into a specific location in the gene for MBP, according to an embodiment of the invention **(GSGGG linker disclosed as SEQ ID NO: 1)**.

Please delete the paragraph on page 12, lines 16-18 and replace it with the following paragraph:

Figure 13 is a schematic diagram illustrating construction schemes and structures of switches isolated from libraries constructed according to the invention **(GSGGG linker disclosed as SEQ ID NO: 1)**.

Please delete the paragraphs on page 22, lines 4-24 and replace it with the following paragraph:

Preferred methods for preparing cyclized genes include a step of adding DNA that codes for a "linker" to link the original N- and C- termini. Any suitable linker sequences can be used for this purpose. Preferred methods of cyclizing a gene utilize linkers such a "DKS linker" (Osuna et al., 2002) or a flexible pentapeptide linker such as a "GSGGG linker" **(SEQ ID NO: 1)** having the amino acid sequence GSGGG **(SEQ ID NO: 1)**. See also Example 1, *infra*, for further details. Generally, the gene fragment of interest (for example a fragment encoding a selected amino acid sequence, such as amino acids 24-286 of the β-lactamase protein), is amplified by a suitable technique such as polymerase chain reaction (PCR) under conditions resulting in flanking of the selected sequence by restriction enzyme site sequences coding for the linkers, and is then cloned into a suitable vector such as pGem T-vector (Promega). Exemplary cloning vectors containing the sequences comprising linkers are indicated in FIG. 1 as pBLA-CP(DKS) or pBLA-CP(GSGGG) **(linker disclosed as SEQ ID NO: 1)**.

The fragments to be cyclized are then released from the cloning vector by digestion with a suitable restriction enzyme and purified, for example by agarose gel electrophoresis. Cyclizing is achieved, for example, by treating with a ligase such as T4 DNA ligase. The cyclized (circular) fragments are subsequently purified and subjected to circular permutation (step iii above). Exemplary circularized genes comprising DKS and GSGGG **(SEQ ID NO: 1)** linkers according to the invention are shown in FIGS. 2 and 3, respectively.

Please delete the paragraph on page 42, lines 15-23 and replace it with the following paragraph:

Additional sequences also can be included as part of the fusion molecule which do not alter substantially the states of the insertion sequence or acceptor sequence portion of the fusion molecule. For example, affinity tag sequences can be provided to facilitate the purification or isolation of the fusion molecule. Thus, His6 tags **(SEQ ID NO: 99)** can be employed (for use with nickel-based affinity columns), as well as epitope tags (e.g., for detection, immunoprecipitation, or FACS analysis), such as myc, BSP biotinylation target sequences of the bacterial enzyme BirA, flu tags, lacZ, GST, and Strep tags I and II. Nucleic acids encoding such tag molecules are commercially available.

Please delete the paragraphs on page 50, line 18 to page 51, line 18 and replace it with the following paragraph:

In order to circularly permute a gene it is generally necessary to include DNA that codes for a linker to link the original N- and C- termini. We chose to test two different linkers. For the first (the "DKS linker"), □-lactamase was randomly circularly permuted by fusing the 5'- and 3'-ends with a DNA sequence coding for the tripeptide linker DKS, previously found in a combinatorial library of linkers to be most conducive for circularly permuting □-lactamase when the new N- and C-termini were located at a specific location (Osuna, Pérez-Blancas et al. 2002). For the second selected linker, (the "GSGGG linker" **SEQ ID NO: 1**), the □-lactamase was randomly circularly permuted by fusing the 5'- and 3'- ends with a DNA sequence coding for the flexible pentapeptide linker GSGGG (SEQ ID NO:1)

Preparation of BLA Insert DNA

The □-lactamase gene fragment bla [24-286] (encoding amino acids 24-286) was selected for this study. DNA coding for amino acids 1-23 was not desired because it codes for the signal sequence that targets ß-lactamase to the periplasm and is not part of the mature, active ß-lactamase. The fragment was amplified by PCR from pBR322 such that it was flanked by *Earl* or *BamHI* restriction enzyme site sequences coding for the linkers described above and cloned into pGem T-vector (Promega) to create pBLA-CP(DKS) (FIG. 2) and pBLA-CP(GSGGG), (FIG. 3) **(linker disclosed as SEQ ID NO: 1)**.

One hundred and thirty micrograms of pBLA-CP(GSGGG) **(linker disclosed as SEQ ID NO: 1)** was digested with 2000 units of *BamHI* and 140 micrograms of pBLA-CP(DKS) was digested with 600 units of *Earl* in the buffers and conditions recommended by the manufacturer of the restriction enzyme. The fragment containing the BLA gene was purified by agarose gel

electrophoresis using the QIAquick™ gel purification kit. This DNA was treated with T4 DNA ligase under dilute concentrations to cyclize the DNA (18 hours at 16 °C with 600 Weiss units of T4 DNA ligase in the presence of 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 25 ug/ml BSA in a total volume of 5.1 ml). The ligation reaction was stopped by incubation at 65 °C for 20 minutes. The DNA was concentrated by vacufuge and desalted using the QIAquick™ PCR purification kit. Circular fragments were purified by agarose gel electrophoresis using the QIAquick™ gel purification kit.

Please delete Table 1., Table 2. and Table 3. on pages 54-55 and replace them with the following tables:

Table 1. Library Statistics.

Insertion site in MBP	Linker in BLA	Library size (Number of transformants with BLA insert).	Number of library members that can grow on 50 µg/ml AMP (see Table 2)	Number of colonies screened for switching (see Table 3)	Number of unique switches found with ≥ 2-fold effect*	Increase in velocity (of nitrocefin hydrolysis in presence of maltose) of best switch
164-165	DKS	0.44×10^6	515	848	2	+97%
	GSGGG	1.05×10^6	361	1248	1	-250%
C-terminus	DKS	1.03×10^6	2414	576	0	
	GSGGG	0.30×10^6	1615	1920	1-4	+234%
Random	DKS	0.41×10^6	191	384	0	
	GSGGG	1.20×10^6	1156	3312	5	+1650%

i.   * ≥ 2-fold change in velocity of nitrocefin hydrolysis in the presence of 5 mM maltose. **(GSGGG linker disclosed as SEQ ID NO: 1**

Table 2. Number of Library Members Capable of Grow on Plates with Ampicillin (With or Without Maltose).

Ampicillin (µg/ml)	Maltose? (5 mM)	T164-165 DKS	T164-165 GSGGG	EE DKS	EE GSGGG	Random DKS	Random GSGGG
5	no	734	878	7052	3510	nd	2458
50	no	394	294	1747	1159	nd	783
200	no	220	nd	1080	298	nd	nd
1000	no	nd	74	nd	nd	nd	60
5	yes	1098	761	8354	4056	nd	1969
50	yes	515	361	2414	1615	191	1156
200	yes	182	240	1525	630	nd	272
1000	yes	nd	88	nd	nd	nd	34

(GSGGG linker disclosed as SEQ ID NO: 1)

Table 3. Number of Library Members Screened (Picked from Plates with Indicated Ampicillin and Maltose Levels).

Ampicillin (μg/ml)	Maltose? (5 mM)	T164-165 DKS	T164-165 GSGGG	EE DKS	EE GSGGG	Random DKS	Random GSGGG
5	no	-	96	-	288	-	96
50	no	-	-	-	-	-	-
200	no	-	-	-	-	-	480
1000	no	-	-	-	-	-	-
5	yes	96	192	-	864	-	768
50	yes	672	576	576	768	384	960
200	yes	80	384	-	-	-	1008
1000	yes	-	-	-	-	-	-

EE = end-to-end (insertion at C-terminus)
(GSGGG linker disclosed as SEQ ID NO: 1)

Please delete Table 4. and the following paragraph on page 56, line 24 to page 57, line 15 and replace them with the following table and paragraph:

Table 4. Switching Effect of Selected BLA-MBP Molecular Switches.

Switch	Sequence	Switching effect*
IFG-5-277	MBP[1-165]-BLA[218-286]-GSGGG-BLA[24-215]-MBP[164-370]	-250%
IFD-5-7	MBP[1-165]-BLA[110-286]-DKS-BLA[24-107]-MBP[164-370]	+96%
IFD-5-15	MBP[1-165]-BLA[168-286]-DKS-BLA[24-170]-MBP[164-370]	+97%
EEG-50-530	MBP[1-370]-BLA[114-286]-GSGGG-BLA[24-112]-GSQQH	+228%
EEG-50-251	MBP[1-370]-BLA[114-286]-GSGGG-BLA[24-114]-K	+234%
RG-5-169	MBP[1-338]-BLA[34-286]-GSGGG-BLA[24-29]-MBP[337-370]	+855%
RG-200-13	MBP[1-316]-BLA[227-286]-GSGGG-BLA[24-226]-S-MBP[319-370]	+1650%

* Percent change in velocity of nitrocefin hydrolysis (50 □M nitrocefin) in the presence of 5 mM maltose in 100 mM sodium phosphate buffer, pH 7.0.
(GSGGG linker disclosed as SEQ ID NO: 1)

Analysis of Purified Switch RG-200-13

A 6xHis tag **(SEQ ID NO: 99)** was added to the C-terminus of RG-200-13 (also termed "RG13" in Examples below) and the fusion was purified as previously described (Guntas and

Ostermeier 2004). The protein was purified to approximately 60% purity. The kinetic constants and binding constants were determined from Eadie-Hofstee plots and Eadie plot equivalents, respectively, using a spectrophotometric assay for nitrocefin hydrolysis. Initial rates for nitrocefin hydrolysis were determined from absorbance at 486 nm monitored as a function of time. The enzyme was incubated at the assay temperature in the absence or presence of saccharide for four minutes prior to performing the assay. All assays contained 100 mM sodium phosphate buffer, pH 7.0. The dissociation constant for maltose was determined using change in velocity of nitrocefin hydrolysis as a signal.

Please delete the paragraph on page 60, line 16 to page 61, line 2 and replace it with the following paragraph:

The portion of the *bla* gene encoding the mature BLA was fused to a sequence coding for a GSGGG linker **(SEQ ID NO: 1)** and containing a *BamH*I site by PCR amplification using the forward primer:
5'-TGCC**GGATCC**GGCGGTGGCCACCCAGAAACGCTGGTG-3'(SEQ ID NO:24)
and the reverse primer
5'- GTCTGA**GGATCC**CCAATGCTTAATCAGTGA-3' (SEQ ID NO:25).

Portions of the primers encoding the GSGGG linker **(SEQ ID NO: 1)** are underlined and the *BamH*I site is highlighted in bold. The PCR product was desalted using Qiaquick PCR purification kit and ligated to the pGEM T-vector to create plasmid pGEMT-BLA. One hundred and fifty □g of pGEMT-BLA was digested with 1000 units of *BamH*I and the DNA fragment that encodes BLA was gel purified using Qiaquick gel purification kit. Eighteen □g of this DNA was cyclized by ligation at 16°C for 18 hours in a reaction volume of 5.1 ml in the presence of ligase buffer (50 mM Tris-HCl, 10 mM MgCl$_2$, 10 mM DTT, 1 mM ATP, 25 □g/ml BSA pH 7.5) and 600 Weiss units of T4 DNA ligase. After heat inactivation of the ligase, the concentrated reaction mixture was desalted and the circular DNA was purified by agarose gel electrophoresis using Qiaquick Gel Extraction kit.

Please delete the paragraph on page 62, lines 18-22 and replace it with the following paragraph:

A GGSGH$_9$ sequence **(SEQ ID NO: 77)** was appended to the sequence of RG13 by PCR amplification with the appropriate primers. The PCR product was cloned between *Nde*I and *Xho*I sites of pET24b (Novagen) to create pET24b-RG13. Mutations I329W and A96W were introduced into pET24b-RG13 by a combination of overlap extension PCR and Quickchange mutagenesis.

Please delete the paragraphs on page 65, line 28 to page 66, line 12 and replace it with the following paragraph:

The fragment of the *BLA* gene coding for the mature protein was circularly permuted in a random fashion (Graf et al. 1996; Ostermeier et al. 2001) and subsequently randomly inserted into a plasmid containing the *E. coli malE* gene that codes for MBP. Figure 6A is a schematic diagram showing the strategy used to make the molecular switch. More particularly, FIG. 6A shows that the fragment of the BLA gene coding for the mature protein (codons 24-286) is flanked by sequences coding for a GSGGG linker **(SEQ ID NO: 1)** (each of which contains a *Bam*HI site). The fragment is excised by digestion with *Bam*HI and cyclized by ligation under dilute DNA concentrations. A single, randomly-located double strand break is introduced by DNaseI digestion to create the circularly permuted library. This library is randomly inserted into plasmid pDIMC8-MBP containing the MBP gene (*malE*) under control of the *tac* promoter (*tacP/O*). The site for insertion in pDIMC8-MBP is created by introduction of a randomly located double-stranded break by digestion with dilute concentrations of DNaseI.

For the random circular permutation of bla [24-286], we fused the 5' and 3' ends by an oligonucleotide sequence that would result in a GSGGG flexible peptide linker **(SEQ ID NO: 1)** between the original N- and C- termini of the protein. This linker was designed to be of sufficient length to connect the termini without perturbing BLA structure.

Please delete the paragraph on page 74, lines 17-21 and replace it with the following paragraph:

Exemplary switches were created by the method having different affinities for maltose. For example switch RG-5-169 (sequence MBP[1-338]-BLA[34-286]-GSGGG-BLA[24-29]-MBP[337-370] **(GSGGG linker disclosed as SEQ ID NO: 1)** was created having a K_d for maltose (> 1 mM) that is much greater than that of RG13 for maltose (1-5 ☐M).

Please delete Table 12. on page 80 and replace it with the following table:

Table 12. Library Statistics for Libraries 2-7.

Library	Library size (number of transformants with *bla* insert).
Library 2 (T164-165/DKS)	0.44×10^6
Library 3 (T164-165/GSGGG)	1.05×10^6
Library 4 (EE/DKS)	1.03×10^6
Library 5 (EE/GSGGG)	0.30×10^6
Library 6	0.75×10^6
Library 7	1.16×10^6

(GSGGG linker disclosed as SEQ ID NO: 1)

Please delete the paragraph on page 85, lines 8-11 and replace it with the following paragraph:

Primer set #1
DIMC8Malfor 5'-GGACCAGGATCCATGAAAATAAAAACAGGT-3'(SEQ ID NO: **76**)
MBP1415rev 5'-GCCGTTAATCCAGATTAC-3'(SEQ ID NO:26)